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A novel member of the serpin superfamily is encoded on a circular plasmid-like DNA species isolated from rabbit cells

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A novel member of the serpin family of serine protease inhibitors is presented. A plasmid-like DNA was isolated from rabbit cells by its homology to the genome of Shope fibroma virus (SFV), a tumorigenic poxvirus of rabbits, and was shown elsewhere to encode a serpin-like protein [(1986) Mol. Cell. Biol. 6, 265–276]. Although significant DNA homology exists between the rabbit plasmid serpin open reading frame and the SFV terminal inverted repeat DNA there is no intact serpin counterpart encoded by this region of the SFV genome. The alignment of the novel plasmid-borne polypeptide with the serpin family of proteins confirms its status within this group.

Shope fibroma virus *Poxvirus* *spc DNA* *Serpin* *Serine protease inhibitor*

1. INTRODUCTION

Members of the serpin (serine protease inhibitors) superfamily (review [1,2]) comprise a variety of distinct but undoubtedly related proteins, not all of which have a known protease inhibitor function. Recently several of the blood plasma protease inhibitors have attracted considerable attention in terms of the therapeutic use of genetically engineered serpins to treat genetic deficiencies, notably that of plasma protein α_1-antitrypsin [3]. Here we describe the serendipitous discovery of a novel member of the serpin superfamily from rabbit cells. This serpin-like protein is derived from the translation of an open reading frame (ORF) on an extrachromosomal plasmid-like DNA species isolated from rabbit cells [4]. This plasmid DNA species was cloned by virtue of its DNA sequence homology to the genome of Shope fibroma virus (SFV), a tumorigenic poxvirus of rabbits [4]. The presence of small polydisperse extrachromosomal circular (spc) DNAs in eukaryotic cells has been observed for a number of years (review [5,6]). The size distribution and number of spc DNAs is found to vary with development, growth state and mitotic capacity of the cell, but the function of these molecules is poorly understood [5–7]. SFV is a tumorigenic leporipoxvirus which is capable of inducing fibromas in rabbits, its natural host [8–10]. Like all poxviruses, SFV replicates in the cytoplasm of infected cells and its large (160 kb) linear ds DNA genome [10] probably encodes all of the proteins required for the replication and transcription of the viral DNA [11,12]. The relationship between SFV and spc DNAs of the host cell is unclear but recombination with these host DNAs may be one way by which the cytoplasmically replicating poxviruses are able to enhance their pool of genetic information [4].

The rabbit sequence described here was discovered when the cloned *Bam*HI fragments of the SFV genome were examined for the presence of host-related sequences which might be implicated in the tumorigenic phenotype of this virus [4]. Unexpectedly, it was found that a 4.8 kb covalently closed circular (CCC) species of DNA, present in both total and Hirt preparations of rabbit (SIRC cell) DNA, hybridized to recombinant plasmids derived from a defined region of the terminal inverted repeats (TIR) of SFV [4]. Analysis of the

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DNA sequence of one clone (pSIC9) which contains 1.9 kb of the plasmid sequence confirmed the homology with SFV TIR sequences found by Southern blotting and revealed the presence of one intact and one truncated ORF [4]. The truncated ORF (ORF-2) was found to be identical to the N-terminus of ORF-T8 in the SFV TIR, but the function of this viral/plasmid gene product has not yet been clarified. The intact ORF (ORF-1) from pSIC9 has no complete counterpart in the SFV TIR, but significant DNA homology exists between the two sequences in this region, although several small gaps plus one of 819 bases must be inserted in order to align these homologies. Translation of this rabbit plasmid ORF-1 yields a 361 amino acid polypeptide which has some homology with human α_1-antichymotrypsin, as determined by computer search through the National Biochemical Research Foundation protein database [4]. Here we report the complete amino acid sequence of the rabbit plasmid ORF-1 and confirm that it represents a novel bona fide member of the serpin superfamily.

2. MATERIALS AND METHODS

The DNA sequence for pSIC9 has been reported [4]. Analysis of the DNA sequence, protein database searches and alignment of the polypeptides were performed using the core library programs of the BIONET computer resource (IntelliGenetics Inc.). A final alignment of the ORF-1 polypeptide to other members of the serpin family utilized the programme of A.M. Lesk, M. Levitt and C. Chothia [1] which uses the Needleman-Wunsch algorithm modified to penalise insertion of gaps in regions of secondary structure based on that of α_1-antitrypsin.

3. RESULTS AND DISCUSSION

The complete amino acid and DNA sequences with the 5′- and 3′-flanking DNA regions of the rabbit plasmid ORF-1 are shown in fig.1. It should

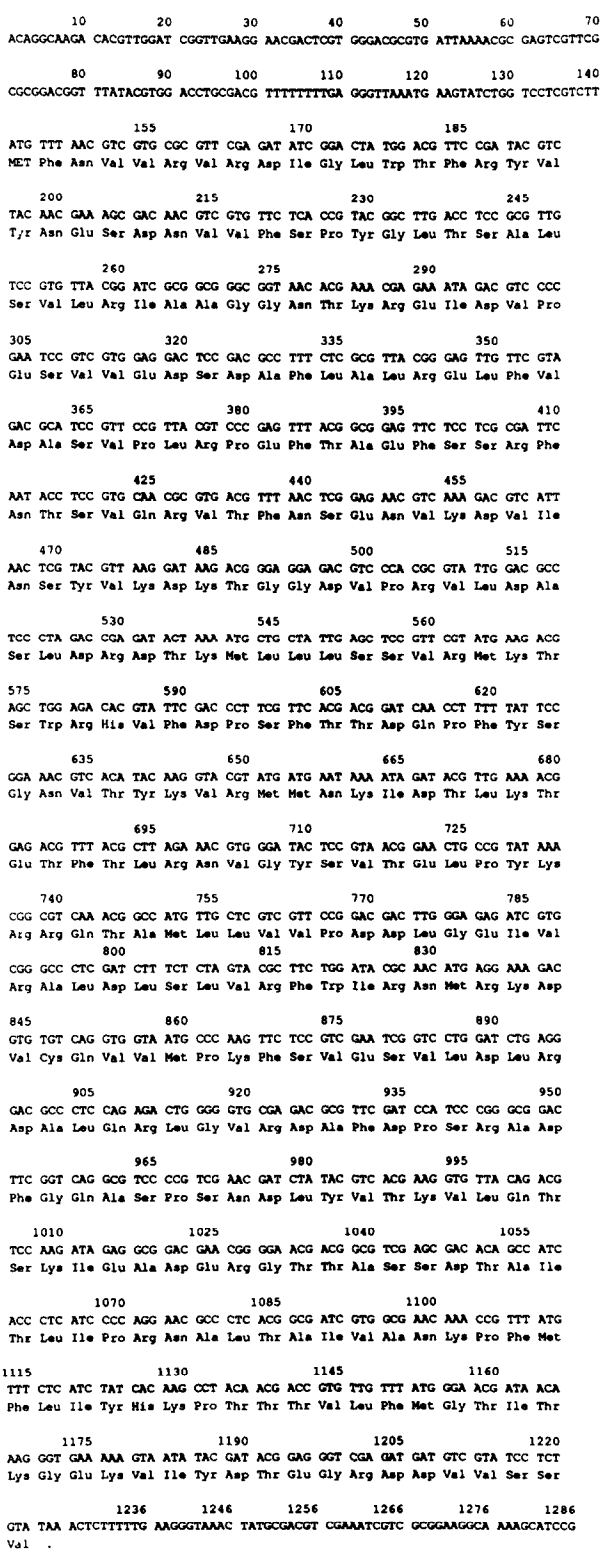

Fig.1. Amino acid sequence of ORF-1, translated from the rabbit plasmid clone pSIC9. The full DNA sequence of clone pSIC9 (complementary strand) is presented in fig.6 of [4] where nucleotide no.1678 corresponds to no.1 of this figure.

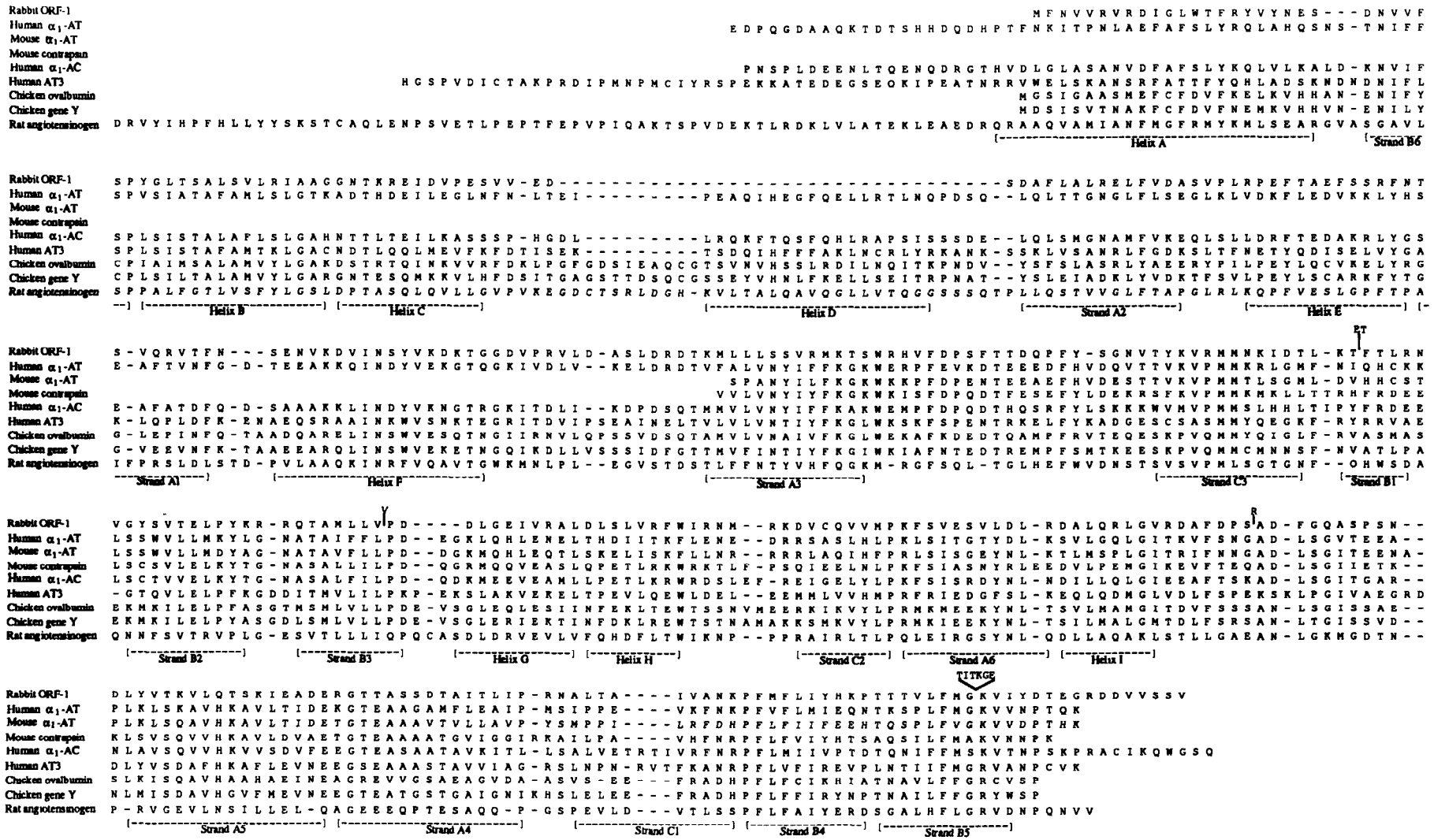

Fig.2. Alignment of the rabbit ORF-1 amino acid sequence with eight members of the serpin superfamily.

be noted that this sequence is translated from a single continuous pSIC9 ORF DNA sequence. In comparison, introns are present within the genes of ovalbumin [13], α_1-antitrypsin [14], angiotensinogen [15] and antithrombin III [16,17], although only in angiotensinogen and antithrombin III are they similarly arranged [15]. The absence of introns from ORF-1 indicates that this rabbit plasmid species may have been originally derived by reverse transcription. In fig.2 the protein sequences of the rabbit plasmid ORF-1, human α_1-antitrypsin (α_1-AT) [18], mouse α_1-AT [19], human α_1-antichymotrypsin (α_1-AC) [20], mouse contrapsin [19], human antithrombin III (AT3) [21], chicken ovalbumin [22], chicken gene Y [13] and rat angiotensinogen [15] have been aligned to minimize the insertion of gaps within regions of secondary structure based on that of α_1-AT. There are 18 residues which are conserved through all the serpins shown and in 133 positions the amino acid is conserved in at least 50% of the proteins. As expected from the alignment of the other serpins there is considerable variation at the N-terminus of the rabbit plasmid ORF-1. An unexpected finding is the complete deletion of the D helix, confirmed by the presence of the conserved sequence in the C helix and the A2 strand that flank each side of the missing D helix. Evidence that the overall conformation is retained at the reactive center is provided by the homologies of the A4 strand region (fig.3). This strand forms the loop that must be present in the native serpins [1] to join the P_1 and P_1' residues of the reactive center. There is strong homology throughout the family at the hinge region of this loop with the typical sequence Glu:polar:Gly:Thr/Ser:Glu

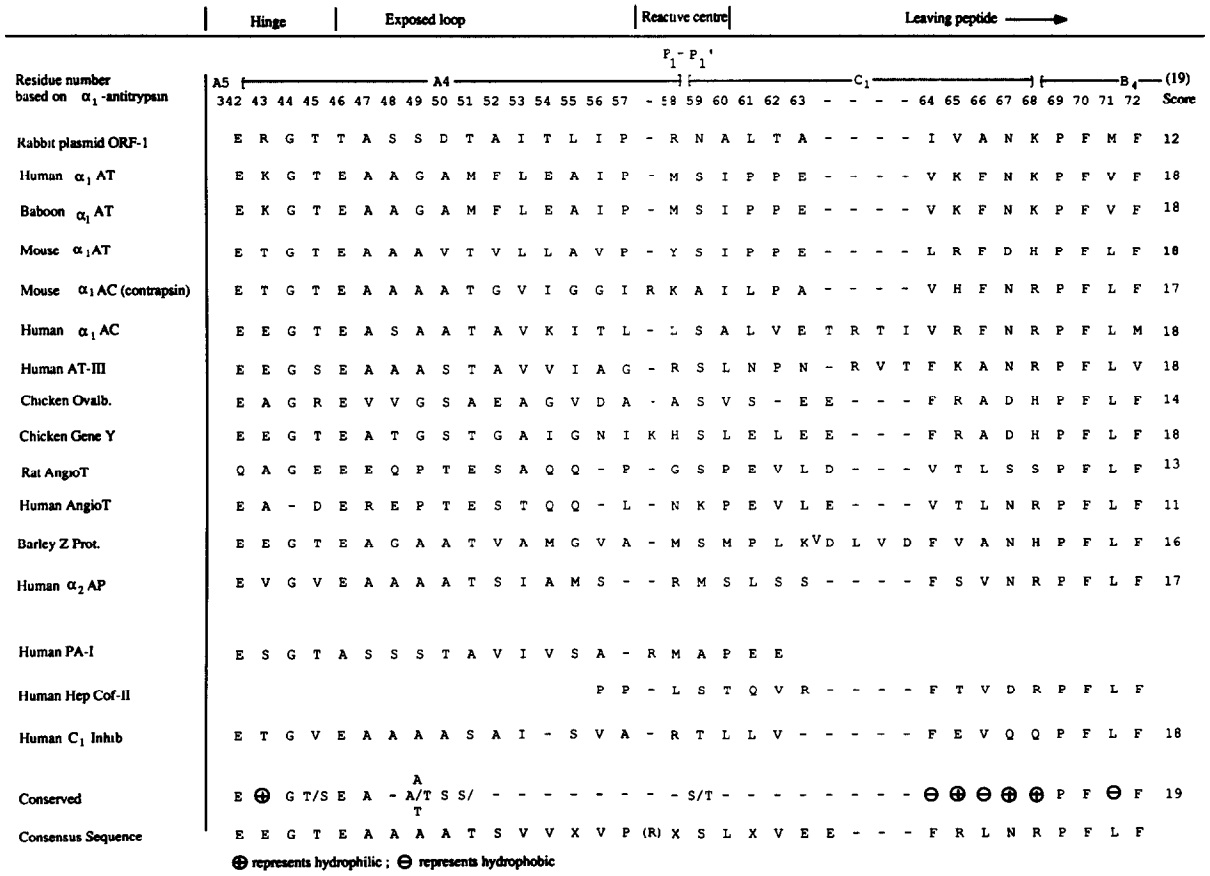

Fig.3. Alignment of the reactive centers of 16 members of the serpin superfamily [1]. The normal cleavage site for the proteinases inhibited by the serpins is after the P_1 residue. Conserved and consensus sequences are indicated.

which is conserved in ORF-1 as Glu:Arg:Gly:Thr:Thr. Furthermore, the ORF-1 sequence has the typical distance of 16 residues between the hinge and the putative P_1 reactive center residue, with the implication that the protein retains the stretched loop conformation necessary for inhibitory function. The common tertiary structure of the serpins is also supported by the even distribution of conserved residues throughout their sequence which can be correlated with the structural features of the α_1-AT model. Examples of this are the conservation of glycine residues at interstrand hinges and salt bridges linking areas of secondary structure. Two Glu-Lys bridges have been maintained in most serpins (fig.2; G7-$B_5$9 and $A_5$16-$A_6$1) with the exception of angiotensinogen, barley protein Z and ORF-1, each of which has lost one complete bridge.

The alignment of the reactive center region of the serpins is shown in fig.3 where more distantly related proteins are included [1,23–27] in addition to a conserved and consensus sequence. The structure of each of the serpins provides a clue to its inhibitory activity and likely target proteinase. The P_1 residue of the reactive center acts as a potential cleavage site for the target enzyme with a specificity that is further increased by the P_2 residue. This is of course an approximate rule but it does allow an estimate as to the target enzymes of serpins of dubious inhibitory activity. Thus the rabbit plasmid ORF-1 fits the description of an Argserpin and if it does have an inhibitory function its target is likely to be a serine protease cleaving after arginine, probably at a Pro:Arg:X sequence.

DNA homology between the SFV TIR and pSIC9 extends across the full 1.9 kb plasmid insert. However, it is obvious that only one of the ORFs (ORF-2, equivalent to SFV ORF-T8) present on pSIC9 has been maintained by SFV [4]. Thus it appears that SFV may have originally acquired a large fraction of the rabbit plasmid sequences but those genes which did not confer a selectable advantage to the virus (including the serpin ORF) were not faithfully maintained in the SFV genome.

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